Pre-Proposal

Potential Food Chain Interactions with Phytoremediation of Strontium-90

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The stem and foliage of Coyote willows (*Salix exigua*) growing along the Columbia River shoreline at 100-N Area of the Hanford Site, and accumulating ^{90}Sr, present both a source of nutrition for various natural herbivores, but also a potential pathway for the isotope to enter the riparian food chain. Because ^{90}Sr generally accumulates in non-consumable tissues (bones, exoskeletons) it has been shown that little, or no, biomagnification of ^{90}Sr isotope occurs in increasingly higher levels of the food chain. However, direct consumers may themselves become contaminated and transport the isotope offsite. To prevent this, a series of engineered barriers will be constructed around the field plot. Large and small animal fencing will control the intrusion of herbivores, such as deer and rodents, and omnivores, such as man. Bird intrusion would be minimized through the use of reflective strips commonly used in orchards. Debris, such as abscised leaves and twigs, will be retained both by the fencing as well as the management practice of removing the foliage twice yearly, before flowering and pollen release (April-May) and prior to leaf drop (September-October). These efforts, however, will have limited effect on mobile phytophagous insects. Management practices such as pesticide application are not acceptable given the proximity of the Columbia River. Therefore, the objective of this task is to evaluate the potential for contamination of insects that may consume plant material grown in soil contaminated with ^{90}Sr.

Scope

There are three major means by which resident and transitory insects may accumulate ^{90}Sr from the tissues of the Coyote willow: consumption of pollen, consumption of sap, and consumption of the foliage and tender shoots. The management practice of scheduled removal of the above ground vegetation prior to flowering will eliminate the possible transfer to pollen collectors (bees, wasps). Direct consumers of the sap and vegetation, however, may still be potentially contaminated. Sap suckers (aphids) feed directly on the transport stream as it comes from the roots and sites of storage. This is a more concentrated material than vegetative tissue. The “honeydew,” or digestive exudates, from the aphids that may fall on other surfaces (e.g., lower leaves and the soil) or becomes a food for other insects (e.g., ants) may be an additional source of contaminant transport. Both the uptake by aphids of ^{90}Sr from the transport streams of willows growing in 100-N Area shoreline sediment, as well as the potential for contaminant loss from the aphid exudates, will be addressed in Subtask 1.

Insects that feed directly on plant tissues (leaves/stems) may also accumulate the contaminant. Further, if they are mobile and are themselves prey of other insects or vertebrates, they may facilitate offsite transport of the contaminant. A primary example of this may be the *Orthoptera* species (grasshoppers), which are present in the Columbia Basin during the summer. They are voracious consumers and are very mobile. The ability of these insects to retain and accumulate ^{90}Sr will be investigated through a series of feeding experiments described in Subtask 2.

Subtask 1 – General Soil/Plant Preparation

There is the potential for a soil to influence uptake of Ca and Sr via its binding specificity toward Ca and Sr. Plant concentrations of Ca and Sr (and by extension ^{90}Sr) have been shown to be related to the soil selectivity coefficient for these elements. Further, concentration coefficients (transfer factors) for Sr were shown to be negatively correlated with extractable soil Ca. Hence, it is imperative that 100-N Area sediment be employed in the feeding study to produce the proper Ca/Sr ratio in the plant tissue. A standardized population of plants and plant material must also be used for all of the feeding experiments. This will be ensured through the use of matched stock willow cuttings taken from the same area and exposed to similar growth conditions in all experiments.

- Soil will be obtained through cores taken along the shoreline at the 100-N Area. This will follow the same procedure as that performed in the 20004 100NC4473 borehole collection. The depth of the cores will encompass material above and below the groundwater level. The material will be sealed in plastic pails for transport to RTL-520.

  – Following transfer to the laboratory, the sediment will be cleaned of large rocks and the remaining material screened to a uniform 2 mm or less size. This will assure uniformity of exposure for the roots during the plant growth period.

  – The sediment will be sub-sampled and replicates taken to determine ^{90}Sr activity on a per gram dry wt. basis. The samples will be extracted with sequential nitric acid washes each taken to dryness. The final extract will be counted using liquid scintillation spectrometry.

  – Coyote willow *(Salix exigua)* cuttings (1-m long) will be obtained from Wildlands, Inc. of Richland, Washington. These originate from alongside the Yakima River in Benton County. The cuttings will be further divided into 30-cm-long pieces. The proximal (closer to the trunk end) will be dipped into a commercial rooting material and kept in distilled water for 14 days until roots and shoots have been initiated from the stem. Thirty Coyote willow saplings (30-cm long and rooted) will be planted into 20-cm diameter pots filled with the screened sediment. The pots will be placed into trays to maintain a water depth of 5 cm at all times on the bottom of the pots. This is to reflect the shallow groundwater encountered at the river shoreline. Saplings will be maintained in a growth chamber at 18-25°C night/day 12-hour light cycle for 90 days.

Subtask 2 – Determination of ^{90}Sr Transfer to Aphids from Labeled Plants

Sucking insects are a common pest in willows. In particular the willow aphid (*Pterochlorus viminalis*) is the most effective in tapping into the vascular system of the plants. They feed directly from the plant phloem found in leaves and in green stems with no secondary growth. The insect is unable to retain all of the sap as it is under pressure in the plant. Because of this the sap drips from the insect's abdomen onto the foliage below and also on to the ground. This provides a potential source of contaminant transfer from the plant to the environment.

- This will be addressed in a feeding experiment where twelve 70- to 90-day-growth plants, grown in the contaminated 100-N Area sediment, will be segregated in the growth chamber. Each will be tented with insect screening. The bottom of the tent structure will be made of plexiglass and the tent will be sealed to this.

- We will obtain a colony of willow aphids native to Washington State from Washington State University (WSU) and maintain them in the greenhouse until needed.

- The insects, a minimum of 50 per plant, will be introduced into the tent surrounding each individual plant in the growth chamber. The insects will be allowed to feed for a period of at least 48 to 72 hours. At the conclusion of this time, the plant will be flushed with CO_2 to anesthetize and euthanize the insects. They will then be collected from the plant, grouped on a per plant basis, and counted. They will then be placed in a container and freeze-dried. A dry weight will be taken to base the activity on a dry weight basis. The insect material will then be ground with a mortar and pestle and powdered CO_2, and transferred to another container. Aliquots of the insect tissue will be extracted and analyzed for ^{90}Sr as described above. The results can be presented both on a per insect as well as pCi/g dry weigt basis.

- The aphid exudates will be collected for the plant and cage surfaces and analyzed to determine the amount of label transfer out of the plant over time. The surfaces will include the plastic bottom of the cage as well as leaf surfaces and the surfaces of the younger green stems. The older tissues with secondary growth (bark) will be impossible to wash effectively. These rinsates will be combined frozen and freeze-dried. The resulting solids will be weighed and analyzed for ^{90}Sr as described above.

- At the end of the 90-day growing season the remaining plants will be removed from the pots, separated into stems and leaves, fresh weights taken, and the tissues placed in individual tared containers filled with powdered dry ice. The containers will then be freeze dried and weighed. The plant material will then be ground in a Wiley Mill (20 mesh) and aliquots will be analyzed for ^{90}Sr to determine the tissue activity (pCi/g). This information will provide data both for the potential transfer of label to the insect, a food source to other insects and birds, as well as reveal the potential of label transfer to the surrounding soil.

^{90}Sr Transfer to Phytophageous (Tissue Eating) Insects – Grasshoppers. There are a number of insect species and life stages (e.g., caterpillars) that directly consume foliage. Insects that consume material such as Sr/Ca in their larval stage will leave much of this behind in their cocoons when they become adults. The most voracious insect is the grasshopper (order *Orthoptera*) these also are the most mobile and, therefore, the greatest candidate for potential off-site transfer of the ^{90}Sr.

  - To address this, we will grow 15 plants in the contaminated sediment as described above for 90 days. Leaves, petioles, and green twigs will be collected from 10 of these and the tissues combined and randomized. The fresh weight will be taken and an aliquot of this plant material will be frozen and stored at -20°C. Half of the remaining tissues (leaves/twigs) will be weighed (fresh weight), freeze dried, weighed again (dry weight) and ground in a Wiley mill with a 20-mesh screen. Aliquots of this dried/ground material will be assayed for ^{90}Sr activity on a pCi/g dry weight basis. The remainder will be weighed and kept as fresh food if supplemental material is needed.

  - Grasshoppers will be obtained from WSU and maintained as a colony in the greenhouse prior to use.

- Nine wire mesh cages (0.5 m x 0.5 m x 0.5 m) will be constructed and placed in the growth chamber. We will then place weighed samples of ground plant material into three of the cages. Weighed samples of the fresh material from the unused plants will be placed in the next three; in the last we will place control plant material grown in the greenhouse. At the completion of a 30-day feeding period, half of the insects in both the exposed and control cages will be euthanized, weighed, and their ^{90}Sr body burden determined. There will be no clearing period; therefore, the insects will be sampled at a gut-loaded condition comparable to being eaten directly after feeding in the field. The amount of ^{90}Sr in the tissue of the grasshoppers will be expressed as a ratio of the amount found in the leaf tissue. The remaining insects will be fed control plant material for an additional 10 days before euthanasia and analysis. This will reflect the potential for residual (incorporated) contaminant transfer for insects that move away from the site prior to being eaten. Data will be analyzed on a weight and per insect basis.

Estimated Cost/time: $190K, 12 to 14 months - perform studies and report results